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Genotype and growing season influence blueberry antioxidant capacity and other quality attributes

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Summary Forty-two blueberry cultivars (thirty-six *Vaccinium ashei*, three *V. ashei* derivative hybrids and three northern highbush standards) were evaluated for their antioxidant capacities and other fruit quality attributes over two growing seasons. Total anthocyanins, phenolic content and antioxidant capacities varied substantially among species and cultivars. 'Early May' (*V. ashei*) had the highest amounts of anthocyanins, phenolics and antioxidant capacity. Cultivars from *V. ashei* hybrid derivatives had lower mean antioxidant components than those from northern highbush standards or *V. ashei*. The antioxidant capacity, as well as total anthocyanins and phenolics, had significant cultivar × year interactions. Correlation coefficient between years for total anthocyanins, total phenolics and oxygen radical absorbance capacity were high with values of 0.86, 0.81 and 0.93, respectively. Similar interactions were observed for soluble solids content (SSC), sugar, titratable acid and organic acids among cultivars both within and across the growing seasons. Correlation coefficients between years for SSC, fructose, glucose and sucrose were 0.78, 0.71, 0.83 and 0.96, respectively. Fructose and glucose were detected as two major sugars with sucrose as a minor constituent. 'Clara' contained the highest amounts of SSC and sugars, while 'Satilla' had the lowest. In general, *V. ashei* cultivars had a higher mean SSC and sugar contents than did the hybrid derivatives or the northern highbush standards. Cultivars of *V. ashei* contained higher malic acid than citric acid, whereas in hybrid derivatives and northern highbush cultivars, citric acid was the predominant organic acid. The diversity in the amount of these fruit quality attributes and antioxidant capacities presents a great opportunity for genetic improvement of blueberries through breeding programmes. The objective of the study was to identify blueberry cultivars with high antioxidant activity and good fruit quality, so they can be used as parents for future blueberry breeding programmes to develop new cultivars with higher antioxidant activity.

Keywords Anthocyanins, antioxidant activities, growing seasons, organic acids, phenolics, sugars, *Vaccinium* species.

Introduction

This study investigated and compared the antioxidant capacities and other quality attributes in forty-two cultivars of blueberry. These cultivars were from three different species including rabbiteye (*Vaccinium ashei* Reade), *V. ashei* hybrid derivatives and northern highbush (*V. corymbosum* L.). Rabbiteye blueberries are native to the south-eastern U.S. and grow best in slightly acidic soil. Northern highbush blueberries are native to eastern North America, suitable to grow in cooler climates and comprise the most common commercial cultivars in the USA and Canada. The *V. ashei* hybrid derivatives have been developed from the crosses of *V. ashei* with *V. constablaei* Gray or *V. ashei* with V. *darrowii* Camp and *V. corymbosum* for later flowering time and earlier fruit ripening than their parents. The crosses have also yielded somewhat hardier rabbiteye hybrid derivatives that may be suitable for the north.

In addition to being a good source of minerals, phenolics, vitamins, dietary fibre and flavonoids,

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blueberries are known to have high antioxidant activity. This is because blueberries are rich in anthocyanins and other phenolic compounds. Antioxidants interact with unstable molecules such as free radicals and may prevent the oxidative damage caused by the free radicals. Antioxidants can also delay or prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidising chain reactions (Seeram *et al*., 2006; Neto, 2007; Kalt *et al*., 2008). Thus, consumption of blueberry fruit may alleviate the development of certain diseases and reduce the risks of some ailments including cancer, heart disease and stroke (Seeram *et al*., 2006; Neto, 2007; Kalt *et al*., 2008). However, considerable variation in antioxidant capacities and other quality attributes exists among cultivars and species of various blueberry genotypes (Prior *et al*., 1998; Kalt *et al*., 2001). This provides an opportunity for selecting and breeding new cultivars for improved nutritional quality.

The high antioxidant activities in fruits are largely attributed to phenolic compounds, such as anthocyanins, and to other flavonoid compounds. The activity is generally considered to be dependent on their structure and content in berries. However, synergistic interactions between phenolics and other components of berry fruits have also been observed to influence the antioxidant activity. Breeding programmes focused for decades predominantly on creating varieties with improved commercial traits, such as large berry size, light berry colour, small scar, desirable flavour, improved firmness and high productivity. Recently, investigations are shifting to emphasise fruit quality and the improvement of the nutritional value, especially to identify phenolic-rich cultivars to breed for enhanced bioactive substances (Scalzo *et al*., 2005) as a desired attribute. This study was undertaken to evaluate the genotypic and seasonal variations of antioxidant capacities and other quality attributes of various blueberry fruit and also to identify cultivars with high antioxidant activity, total anthocyanins, phenolic content, sugars and organic acids for use as parents in future blueberry breeding programmes.

Materials and methods

Fruit sample handling and preparation

Blueberry fruit (*Vaccinium* species) used in this study were grown in USDA-ARS plots at the Marucci Center for Blueberry and Cranberry Research and Extension, Chatsworth, NJ, USA. Fully mature (100% blue) blueberries were hand harvested at overall fruit ripeness from 45% to 100% with the most typical value being approximately 60%. Approximately 500–900 g of fruit was harvested per genotype from test plots of two plants of each cultivar. Forty-two different genotypes were sampled and used for this study. This included thirty-six rabbiteye (*V. ashei*) blueberry cultivars along with three *V. ashei* hybrid derivatives, and three northern highbush blueberry standards as listed in Tables 1–3. The *V. ashei* hybrid derivative 'Pink Lemonade' is a pink-fruited cultivar released as an ornamental also included in this study (Ehlenfeldt & Finn, 2007). The fruit was sorted to eliminate damaged, shrivelled and unripe fruit and to select for uniform size and colour. Berries were initially frozen in a −70 °C freezer, then transported to Beltsville with freezer packs in a cooler and ultimately stored at −80 °C until they were used for analysis.

Triplicate composite 5 g blueberry fruit samples were extracted four times with 50% acetone using a Polytron (Brinkmann Instruments, Inc., Westbury, NY, USA). The homogenised samples from the acetone extracts were then centrifuged at 14 000 ***g*** for 20 min at 4 °C. The supernatants were combined, for a final volume of 50 mL, and this was transferred to vials and stored at −80 °C until analysis for antioxidant activity, total phenolics and total anthocyanins. All analyses were replicated three times.

Oxygen radical absorbance capacity (ORAC) assay

The automated sample preparation was performed using a Precision 2000 instrument (Bio Tek Instrument, Winooski, VT, USA). The ORAC assay was carried out using a high-throughput instrument platform consisting of a robotic eight-channel liquid-handling system and a microplate fluorescence reader (FL800, Bio Tek) following the protocol previously described (Huang *et al*., 2002).

Measurement of total anthocyanins and phenolics

Total phenolics in the fruit extract were determined with Folin–Ciocalteu reagent. Because the Folin–Ciocalteu assay could be affected by several interfering substances, such as sugars, aromatic amine, sulphur dioxide, ascorbic acid, organic acids, Fe (II), as well as some non-phenolic organic substances (Box, 1983), a solid-phase extraction (SPE) procedure was used to remove such water-soluble compounds from fruit extract samples. Five milli litres from the above acetone-formic acid extracts was concentrated to 1 mL using a Buchler Evapomix (Fort Lee, NJ, USA) in a water bath at 30 °C. The concentrated samples were dissolved in 4 mL of acidified water (3% formic acid) then passed through a C_{18} Sep-Pak cartridge (Waters, Milford, MA, USA), which was previously activated with methanol followed by water and 3% aqueous formic acid. The interfering substances such as sugars, ascorbic acid, organic acids and non-phenolic organic substances that react with Folin–Ciocalteu were all passed through C_{18} Sep-Pak column. Anthocyanins and other phenolics were retained by the column and then recovered with 5.0 mL of

Table 1 The analysis of variance results for variables: (A) total anthocyanins, total phenolics and oxygen radical absorbance capacity (ORAC). (B) soluble solids (SSC), fructose, glucose and sucrose (C) titratable acidity (TA %), citric acid and malic acid

(A) Analysis of variance for Table 2

		Total anthocyanins		Total phenolics		ORAC	
Source	**DF**	***F*-value**	***P*-value**	***F*-value**	***P*-value**	***F*-value**	***P*-value**
Cultivar (Cv)	29	2717.5	<0.0001	7319.4	<0.0001	158.40	<0.0001
Year	1	5329.8	<0.0001	1833.2	<0.0001	11.14	0.0012
Cv × year	29	323.5	<0.0001	341.7	<0.0001	2.00	0.0068

(B) Analysis of variance for Table 3

		SSC		Fructose		Glucose		Sucrose	
Source	**DF**	***F*-value**	***P*-value**	***F*-value**	***P*-value**	***F*-value**	***P*-value**	***F*-value**	***P*-value**
Cultivar (Cv)	31	125.30	<0.0001	46.94	<0.0001	11.55	<0.0001	185.76	<0.0001
Year	1	149.15	<0.0001	1932.28	<0.0001	263.46	<0.0001	14.22	0.0006
Cv × year	31	12.67	<0.0001	4.55	<0.0001	0.59	0.9377	1.06	0.4078

(C) Analysis of variance for Table 4

		TA (%)			Citric acid		Malic acid	
Source	**DF**	***F*-value**	***P*-value**	**DF**	***F*-value**	***P*-value**	***F*-value**	***P*-value**
Cultivar (Cv)	32	340.20	<0.0001	40	2311.2	<0.0001	422.98	<0.0001
Year	1	19.02	<0.0001	1	13.0	<0.0001	7.43	<0.0001
Cv × year	32	15.88	<0.0001	40	7.95	<0.0001	11.32	<0.0001

acidified methanol containing 3% formic acid. Total phenolics were then determined with Folin–Ciocalteu reagent by the method of Slinkard & Singleton (1977) using gallic acid as standard. Results were expressed as milligrams gallic acid equivalent, in the blueberry extract, per 100 g fresh weight.

Total anthocyanin content in tissue extracts was determined using the pH differential method (Cheng & Breen, 1991). Absorbance was measured in a Shimadzu spectrophotometer (Shimadzu UV-160, Columbia, MD, USA) at 510 and 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}]$ with a molar extinction coefficient of cyanidin-3-glucoside (26 900) (Wrolstad *et al.*, 2005). Results were expressed as milligrams of cyanidin-3-glucoside equivalent per 100 g of fw.

Soluble solids content (SSC), sugars, titratable acidity (TA) and organic acids assay

Soluble solids content of the fruit was determined at 20 °C on a digital refractometer (PR-101; Spectrum Technologies, Plainfield, IL, USA). TA was determined by diluting each 5-mL aliquot of blueberry juice to 100 mL with distilled water and adjusting the pH to 8.2 using 0.1 N NaOH. Acidity was expressed as per cent of citric acid equivalent. For sugar analysis, five grams of blueberries was homogenised with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY, USA) in imidazole buffer (20 mM, pH 7.0). The extracts were centrifuged, and the supernatants were dried in vacuo in vials that were used during derivatisation. Procedures described by Wang *et al.* (1987) were modified for the derivatization of sugars and organic acids. A known amount of β-phenyl-D-glucopyranoside was included in all samples as an internal standard. One millilitre of Trisil reagent (Pierce, Rockford, IL, USA) was mixed vigorously with each sample and then heated at 75 °C for 30 min. After silylation, 1 μL of each derivatised sample was injected into a Hewlett-Packard 5890 gas chromatograph (Palo Alto, CA, USA) equipped with a flame ionisation detector and a 25-m cross-linked methyl silicon gum capillary column (Hewlett-Packard, HP-1, 0.2 mm i.d., 0.33 μm film thickness) and using helium as carrier gas. Temperatures were as follows: injector, 250 °C; detector, 275 °C; column temperature, programmed to increase from 100 to 250 °C at 10 °C min-1 and then held constant at 250 °C for 23 min. Organic acids were analysed after extraction with imidazole buffer (20 mM, pH 7.0) and purification with a Baker-10 solid-phase extraction system. Supernatants from the extract were passed through quaternary amine columns,

Table 2 Genotypic and seasonal variations of total anthocyanins, total phenolics and antioxidant capacities in fruit of forty-two blueberry cultivars (thirty-six *V. ashei*, three *V. ashei* derivative hybrids and three northern highbush standards)

	Total anthocyanins				Total phenolics				ORAC			
	mg 100 g^{-1} fw				mg 100 g^{-1} fw				μmol TE g^{-1} fw			
Blueberry cultivar	**2008**		**2009**		**2008**		**2009**		**2008**		**2009**	
Vaccinium ashei Reade (rabbiteye)												
Alapaha	185.5 r*		–		236.2 r		–		49.8 i		–	
Aliceblue	441.1 b	α†	351.9 c	β	404.3 g	α	397.0 ef	α	82.4 cd	α	80.5 cd	α
Austin	330.1 i	α	211.1 j	β	272.8 p	α	275.0 n	α	45.2 i	α	48.8 kl	α
Baldwin	289.2 m	α	192.1 kl	β	325.8 n	α	298.0 m	β	63.6 fgh	α	63.6 gh	α
Beckyblue	153.4 t		–		135.9 u		–		33.8 j		–	
Black Giant	–		283.7 g		–		382.4 ghi		–		71.4 e-h	
Bluebelle	265.4 n	α	206.8 j	β	227.0 r	α	230.0 o	α	49.9 i	α	51.7 jk	α
Bluegem	401.6 d		–		524.1 b		–		92.3 b		–	
Bonita	245.8 o		–		268.6 q		–		62.4 h		–	
Brightwell	299.5 kl	α	222.5 ij	β	301.9 o	β	352.0 j	α	60.4 h	α	60.7 g–k	α
Briteblue	295.7 kl	α	285.4 g	β	391.7 hi	α	384.0 ghi	β	76.8 cde	α	75.7 c–f	α
Callaway	348.1 h	α	315.3 f	β	325.5 n	β	373.0 i	α	69.8 e–h	β	74.8 c–h	α
Centurion	425.4 c	α	408.5 b	β	515.5 b	β	606.0 a	α	81.3 cd	β	93.2 b	α
Chaucer	267.8 n		–		207.0 s		–		61.2 h		–	
Choice	330.3 i	α	335.0 c–f	α	347.8 l	β	380.0 hi	α	76.0 de	α	75.5 c–f	α
Clara	360.9 g	β	398.7 b	α	471.1 d	α	460.0 c	β	90.7 b	α	96.3 b	α
Climax	424.5 c	α	404.5 b	β	489.0 c	β	557.0 b	α	86.0 bc	α	93.3 b	α
Coastal	224.2 q	α	132.0 n	β	298.3 o	α	276.0 n	β	49.4 i	α	53.2 jk	α
Delite	127.3 u	β	146.7 m	α	183.6 t	β	208.6 p	α	39.2 j	α	42.4 l	α
Early May	475.9 a	α	452.3 a	β	660.1 a	α	622.0 a	β	118.7 a	α	114.8 a	α
Ethel	398.8 e	α	338.0 cd	β	364.5 k	α	398.0 ef	α	73.6 c–g	α	70.0 e–h	α
Garden Blue	369.4 f	α	322.5 def	β	340.2 lm	β	347.0 jk	α	67.6 e–h	α	71.6 e-h	α
Homebell	398.5 e	α	344.5 c	β	400.9 gh	α	406.0 ef	α	75.6 c–g	α	72.3 d–g	α
Ira	324.3 i	β	337.3 cd	α	336.7 m	β	393.0 fg	α	70.2 efg	α	69.9 e–h	α
Menditoo	314.4 j	β	341.6 cd	α	380.4 j	α	393.1 fg	β	68.7 e–h	α	69.3 e–h	α
Montgomery	290.9 lm	α	178.5 kl	β	281.1 p	β	328.0 kl	α	61.4 gh	α	64.5 f–j	α
Myers	329.1 i	α	293.1 g	β	467.1 d	β	548.0 b	α	83.1 cd	α	85.7 bc	α
Owen	403.8 d		–		443.9 e		–		94.1 b		–	
Powderblue	232.5 p	α	240.3 hi	α	424.4 f	α	407.0 e	β	64.2 e–h	α	68.4 e–h	α
Premier	313.7 j	α	220.9 j	β	360.8 k	β	420.0 d	α	67.3 e–h	α	67.8 e–h	α
Satilla	321.5 i		–		315.8 n		–		60.2 h		–	
Sout	358.4 g	α	333.1 c–f	β	382.2 ij	β	394.0 fg	α	68.3 e–h	α	68.6 e–h	α
Suwanee	344.4 h	α	252.0 hi	β	338.5 m	α	302.0 lm	β	61.6 gh	α	62.4 h	α
Tifblue	318.8 j	α	320.6 ef	α	364.9 k	β	380.0 hi	α	76.7 cde	α	76.2 c–f	α
Walker	252.4 o	α	162.2 l	β	253.7 q	α	218.0 op	β	49.1 i	α	52.0 jk	α
Windy	356.0 g		–		284.1 p		–		68.0 e–h		–	
Woodard	399.6 e	α	279.9 g	β	385.9 ij	β	455.0 c	α	80.8 cd	α	77.5 c–f	α
Mean	322.7		286.6		353.1		385.9		68.9		71.5	
Vaccinium ashei hybrid derivatives												
Pearl River	62.2 v	β	92.0 o	α	132.3 u	β	202.0 p	α	38.4 j	α	39.1 l	α
Pink Lemonade	15.7 w		–		87.1 v		–		37.3 j		–	
Snowflake	168.0 s		–		235.7 r		–		52.8 i		–	
Mean	82.0		92.0		151.7		202.0		42.8		39.1	
Vaccinium corymbosum L. (northern highbush)												
Bluecrop	180.3 r	α	173.3 l	α	136.4 u	β	297.0 m	α	46.0 i	β	50.7 kl	α
Duke	242.1 o	α	165.2 l	β	189.0 t	β	275.2 n	α	45.7 i	α	50.6 kl	α
Elliott	406.9 d	α	349.2 c	β	450.1 e	α	384.5 l	β	78.5 cd	α	78.4 c–f	α
Mean	276.4		229.2		258.5		318.9		56.7		59.9	

*Cultivar means within year with different letters are different at the 0.05 significance level ($n = 3$).
†α and β indicate differences at the 0.05 significance level between years within a cultivar.

which were previously conditioned with hexane and methanol. The samples were then eluted from the columns with 0.1 N HCI. The eluates were concentrated to dryness in vacuo in derivatised vials. Procedures of derivatisation and chromatography for organic acids were the same as those for sugars except that column temperature was held at 180 °C for 3 min, then increased to 250 °C at 10 °C min-1 and held at 250 °C for 12 min. The sugars and organic acids were quantified by comparison with derivatized standards.

Statistical analysis

The variables were analysed as two-factor linear models using PROC MIXED (SAS Institute Inc, 2010) with cultivar and year as the factors. Only cultivars that had blueberry samples from both years were included in the analysis. The assumptions of the model were checked, and the variance grouping technique was used to correct for heterogenous variances. Means comparisons were done with Sidak adjusted *P*-values so that the experiment-wise error was 0.05.

Results and discussion

Total anthocyanins, phenolics and antioxidant capacities

The highest total anthocyanin content among the forty-two cultivars evaluated was in 'Early May' (Table 2). This amount was approximately thirty times greater than that in the pink-fruited cv. pink lemonade, which had the lowest total anthocyanin content. When analysed, significant differences in total anthocyanin content were found among various cultivars within years and also between the 2 years when cultivars were individually compared (Table 1A). Blueberry cultivars with high anthocyanins are desirable because anthocyanins have been reported to help reduce damage caused by free radical activities, such as low-density lipoprotein oxidation, platelet aggregation and endothelium-dependent vasodilatation of arteries (Heinonen *et al*., 1998). 'Early May' also had the highest level of total phenolics and 'Pink Lemonade' (pink fruited) had the lowest level of total phenolics (Table 2). In general, the cultivars from hybrid derivatives tended to have lower phenolic levels than cultivars from *V. ashei* or northern highbush standards. Like anthocyanin content, significant differences in total phenolic content were found among various cultivars within years, but also between the 2 years for many cultivars (Table 1A).

Likewise, antioxidant capacities were significantly different among cultivars and between seasons (Tables 2 and 1A). 'Beckyblue' had the lowest antioxidant capacity, while 'Early May' had the highest antioxidant capacity. Similar to total phenolics and total anthocyanins, cultivars in hybrid derivatives generally had lower antioxidant capacities than those from *V. ashei* or northern highbush standards (Table 2). Howard *et al*. (2003) reported that ORAC values of eighteen blueberry cultivars ranged from a low 20.5 μmol TE g^{-1} fw in 'Magnolia' to a high 60.3 μmol TE g^{-1} fw in US-497, a 2.9-fold difference. Other studies reported that ORAC values among blueberry genotypes varied 1.8-fold (Kalt *et al*., 1999), 2.5-fold (Prior *et al*., 1998), 3.3-fold (Sellapan *et al*., 2002), 4.7-fold (Connor *et al*., 2002), 5.2-fold (Moyer *et al*., 2002) and 6.8-fold (Ehlenfeldt & Prior, 2001). These variations are strongly dependent on many factors, such as genotypes, climate, sample handling, etc. Moyer *et al*. (2002) evaluated thirty genotypes of nine species of *Vaccinium* and found *V. ashei* had the highest antioxidant capacity with ORAC values from 110.8 to 130.7 μmol TE g^{-1} fw and ferric reducing antioxidant power (FRAP) values from 127.1 to 157.3 μmol TE g^{-1} fw, followed by *V. angustifolium* Ait., *V. ovatum* Pursh and *V. parvifolium* Smith. These indicate that ample genetic variation exists for exploitation by plant breeders. In this study, the correlation was significant at $P \leq 0.05$ for ORAC vs. anthocyanins ($r = 0.61$) and ORAC vs. total phenolics ($r = 0.89$). Moyer *et al*. (2002) found that the correlation coefficients of total anthocyanins, total phenolics and ORAC or FRAP vary among different genotypes of *Vaccinium, Rubus* and *Ribes*. Prior *et al*. (1998) reported that the correlation coefficient of ORAC and total phenolic content was higher than that of ORAC and anthocyanin in fruit of *Vaccinium* species. Connor *et al*. (2002) showed in blueberries that total phenolics correlated better with antioxidant activity ($r = 0.82$) than did total anthocyanins ($r = 0.73$). Anthocyanins and phenolics are secondary plant metabolites. They protect the plant against damaging photodynamic reactions by quenching the excited state of active oxygen species (Lewis, 1993). In general, the correlation coefficient of antioxidant activity and phenolic content is better than that of ORAC value and anthocyanin. These results suggest that the antioxidant activity of fruit is mainly derived from the contribution of phenolics in fruits. Antioxidants are effective in preventing or alleviating various human diseases caused by oxidative stress (Lewis, 1993). Thus, consumption of fruits high in antioxidants could significantly reduce the incidence and mortality rates of cancer, cardiovascular disorders and other degenerative diseases caused by oxidative damage (Ames *et al*., 1993; Cao *et al*., 1998). Selection and breeding for high antioxidant capacity is, therefore, an important goal of fruit research.

Variations of SSC, sugars, TA and organic acids

Sugars and organic acids have an important impact on the sensory quality of fruit. The general flavour selection criteria for fruits are a combination of high sweetness

and high acidity. Although not all blueberries with high SSC will necessarily be of good quality, the absence of high SSC makes good quality unlikely. Significant differences in fruit SSC and TA content were found among cultivars within years and also between years for many cultivars (Tables 3 and 1B). SSC among the forty-two cultivars of blueberries ranged from 12.5% to 18.9% and TA ranged from 0.31% to 1.14%. 'Clara' had the highest SSC and 'Windy' and 'Satilla' had the lowest SSC; 'Ethel' had the highest TA, meanwhile 'Callaway' and 'Pink Lemonade' (pink fruited) had the lowest TA (Tables 3 and 4). Prior *et al.* (1998) reported that in six northern highbush blueberry fruit, SSC was in the range of 10.0–19.0%, TA was 0.22–0.73% and in six rabbiteye blueberries SSC ranged from 10.0 to 17.0 and TA ranged from 0.26% to 0.85% depending on cultivars and berry grown location. Perkins-Veazie *et al.* (1995) also found 10 southern highbush blueberry cultivars, the SSC ranged from 9.0% to 11.5% and TA ranged from 0.54% to 1.13.

Fructose and glucose were the predominant sugars comprising 61.6% of the total SSC in fruit. Significant differences in fructose and glucose were found among cultivars within years, as well as between the 2 years for many individual cultivars (Table 3 and 1B). The mean sugar composition of the 42 cultivars was as follows: fructose 31.0%, glucose 27.8% and sucrose 2.7%. Fruit contained lower sucrose concentrations compared to fructose and glucose. The low sucrose content in the fruit is probably due to enzymatic hydrolysis. Fructose varied from 47.4 mg g^{-1} fw (2008) in 'Satilla' to 72.9 mg g^{-1} fw in 'Clara' (2009) in our study (Table 3). Glucose varied from 26.6 mg g^{-1} fw to 486.1 mg g^{-1} fw (Table 3). The cultivar 'Clara' had the highest fructose, glucose and sucrose, whereas 'Windy' and 'Satilla' had the lowest. Quality attributes such as SSC, TA and organic acids varied considerably in various cultivars and in different seasons (Tables 3 and 4). SSC is a rough indicator of the sweetness of the fruit. SSC primarily measures the total amount of sugars, even though soluble pectins, titratable acids, etc. also contribute to the SSC value. Blueberries have relatively high SSC compared with other fruits. This may be because blueberries have relatively high content of fructose and titratable acids (Tables 3 and 4). Fructose is more than 1.5 times sweeter than sucrose and is the sweetest of all naturally occurring carbohydrates and is the most water soluble of all sugars (Hanover & White, 1993). Although not as sweet as fructose, glucose is the primary energy source of the three sugars and is used as a precursor for the synthesis and production of several important substances such as vitamins, proteins and lipids. Sucrose was also present in blueberries, but in less amounts. There were significant ($P \leq 0.05$) correlations between SSC and fructose, glucose and sucrose with correlation coefficient (r) values at 0.75, 0.81 and 0.71, respectively.

Organic acids are minor components of blueberries, but they are important attributes of flavour, in combination with sugars, that have an impact on sensory quality. There were distinct differences among cultivars, and this may reflect the genetic origins of the cultivars examined. Significant differences in organic acids were found among various cultivars within years and also between years for many cultivars (Table 4 and 1C). In rabbiteye blueberries (*V. ashei*), malic acid was higher than citric acid, whereas in *V. ashei* hybrid derivatives and northern highbush standards (*V. corymbosum*), citric acid was the predominant organic acid (Table 4). Ehlenfeldt *et al.* (1994) had reported previously that highbush blueberries clones were characterised by high citric acid with values that ranged from 38% to 90% of organic acids, whereas, rabbiteye cultivars contained malic acid as their main component, and no clone had citric acid $>22\%$. In this study, 'Woodard' contained the highest malic acid and 'Elliott' had the lowest. Northern highbush 'Bluecrop', 'Duke' and 'Elliott' all contained high citric acid. The total organic acid level was significantly ($P \leq 0.05$) correlated with titratable acidity ($r = 0.84$ for all 42 cultivars; $r = 0.95$ for thirty-six rabbiteye and three cultivars of *Vaccinium* hybrids; $r = 0.99$ for three northern highbush blueberry cultivars). The ratios of SSC to TA ranged from 11.8 to 62.7 with 'Callaway' having the highest SSC/TA ratio and 'Satilla' having the lowest. Usually, high sugar and high acid content are required for constituting high fruit quality. However, different proportions of sugars and organic acids affect the flavour and sensory quality of fruit. For example, fructose contributes to sweetness, malic acid increases the likelihood of tartness and citric acid enhances the acidic and sour taste of the fruit.

Year-to-year variation in fruit composition

Seasonal differences had a large impact on the accumulations of anthocyanins, phenolics, antioxidants, sugars and organic acids. For all components evaluated, except glucose and sucrose, there were highly significant cultivar × year interactions (Table 1).

Despite interaction effects, there were high correlation coefficients (r) between years for total anthocyanins, total phenolics and ORAC with values of 0.86, 0.81 and 0.93, respectively (significant at $P \leq 0.05$). In general, the total anthocyanin values were higher in 2008 than in 2009; however, the reverse was true for total phenolics. ORAC values between years showed few significant differences (Table 2). Among the thirty-two cultivars where samples were available for both years, the average change in rank for total anthocyanins, total phenolics and ORAC between years were 3.3 (range, 0–12), 3.4 (range, 0–17) and 1.0 (range, 0–4), respectively, thus showing that ORAC was the most stable across years among these three parameters (Table 2).

Table 3 Genotypic and seasonal variations of soluble solids content (SSC), fructose, glucose and sucrose content in fruit of forty-two blueberry cultivars (thirty-six *V. ashei*, three *V. ashei* derivative hybrids and three northern highbush standards)

	SSC				Fructose				Glucose				Sucrose			
	%				mg g^{-1} fw				mg g^{-1} fw				mg g^{-1} fw			
Blueberry cultivar	**2008**		**2009**		**2008**		**2009**		**2008**		**2009**		**2008**		**2009**	
Vaccinium ashei Reade (Rabbiteye)																
Alapaha	16.2 ghi*		–		58.7 f–k		–		39.6 b–e		–		3.5 klm		–	
Aliceblue	17.0 def	α †	15.9 efg	β	67.2 bc	α	52.3 bcd	β	35.8 h–l	α	41.8 ijk	β	4.8 gh	α	4.5 g	α
Austin	17.5 abc	α	16.2 cde	β	62.7 de	α	48.5 def	β	38.4 c–g	α	44.6 fgh	β	5.6 ef	α	5.2 ef	α
Baldwin	17.9 abc	α	16.7 bcd	β	71.6 a	α	55.9 ab	β	40.0 bcd	α	46.9 cd	β	7.9 b	β	7.4 ab	α
Beckyblue	17.4 abc		–		66.9 bc		–		38.9 b–g		–		5.8 ef		–	
Black Giant	–		16.2 cde		–		48.2 def		–		45.0 efg		–		5.2 ef	
Bluebelle	18.1 ab	α	18.3 a	α	71.3 ab	α	60.6 a	β	39.5 b–e	α	49.8 a	β	6.9 c	α	7.0 c	α
Bluegem	18.2 ab		–		69.8 ab		–		43.4 a		–		7.2 c		–	
Bonita	16.5 efg		–		62.2 d–g		–		35.7 i–l		–		4.2 hij		–	
Brightwell	17.3 cde	α	15.2 gh	β	62.1 d–g	α	45.7 d–g	β	41.2 ab	α	45.4 def	α	4.8 gh	β	4.2 g	α
Briteblue	16.1 ghi	α	14.7 ijk	β	56.5 e–o	α	42.9 e–o	β	36.8 f–g	α	41.9 ijk	α	3.8 ijk	α	3.5 hi	α
Callaway	16.5 efg	α	16.7 bcd	α	56.7 j–m	α	47.9 def	β	36.5 g–k	α	46.3 de	β	4.3 hij	α	4.4 g	α
Centurion	15.5 ijk	α	14.8 ijk	α	55.8 j–o	α	44.3 j–o	β	36.5 g–k	α	43.4 ghi	β	3.0 l-p	α	2.8 j–m	a
Chaucer	18.0 ab		–		67.1 bc		–		40.9 bc		–		6.2 de		–	
Choice	17.7 abc	α	17.8 abc	α	64.0 b–h	α	53.8 bcd	β	38.2 d–i	α	48.2 bc	β	5.9 e	α	6.0 d	α
Clara	18.9 a	α	17.1 bc	β	72.9 a	α	55.0 ab	β	43.3 a	α	48.9 ab	α	8.5 a	β	7.7 a	α
Climax	17.0 def	α	16.0 cde	α	59.1 d–k	α	46.5 d–k	β	39.6 b–e	α	46.8 cd	P	5.9 e	α	5.5 e	α
Coastal	15.7 hij	α	15.0 ghi	α	56.5 e–o	α	45.1 e–o	β	33.6 l–o	α	40.2 k	β	3.7 jk	α	3.6 h	α
Delite	15.4 ijk	α	15.1 ghi	α	59.0 e–k	α	48.5 def	β	33.8 l–o	α	41.7 jk	β	2.9 m–q	α	2.9 jkl	α
Early May	17.7 abc	α	17.4 abc	α	61.3 d–i	α	50.2 cd	β	39.2 b–f	α	48.1 bc	β	7.2 c	α	7.1 bc	α
Ethel	16.7 bcd	α	15.9 def	α	57.4 g–l	α	45.8 f–k	β	37.3 e–j	α	44.6 e–h	β	3.8 ijk	α	3.6 h	α
Garden Blue	14.9 km	α	15.1 ghi	α	53.4 m–p	α	45.1 g–l	β	32.8 mno	α	41.6 jk	β	2.6 opq	α	2.7 klm	α
Homebell	15.7 hij	α	15.2 gh	α	60.3 d–k	α	48.8 def	β	33.8 l–o	α	41.1 k	β	4.5 h	α	4.4 g	α
Ira	14.3 jkl	α	13.1 nop	β	52.1 op	α	39.7 p	β	29.1 p	α	33.3 n	α	3.2 k–o	α	2.9 jkl	α
Menditoo	16.3 fgh	α	14.9 ijk	β	62.4 def	α	47.5 def	β	33.3 l–o	α	38.1 l	α	3.7 jk	α	3.4 hi	α
Montgomery	13.3 no	α	14.0 klm	α	49.9 p	α	44.1 no	β	31.3 o	α	41.4 k	β	2.5 pq	α	2.7 klm	α
Myers	17.4 abc	α	16.4 bcd	β	63.8 cd	α	50.2 cd	β	39.0 b–g	α	46.1 def	β	6.7 cd		6.3 d	
Owen	17.9 abc		–		63.4 cd		–		37.1 e–j		–		6.9 c		–	
Powderblue	16.4 efg	α	15.4 fg	β	58.3 f–k	α	45.8 f–k	β	35.2 j–m	α	41.5 k	β	5.2 fg	α	4.9 f	α
Premier	15.2 jkm	α	14.9 ijk	α	54.9 k–o	α	44.9 klm	β	37.2 e–j	α	45.6 def	β	4.4 hi	α	4.3 g	α
Satilla	12.5 o		–		47.4 q		–		26.6 q	α	–		1.8 r		–	
Southland	14.2 mn	α	13.2 nop	β	57.1 h–n	α	44.2 h–n	β	32.8 mno	α	38.2 l	α	2.6 opq	α	2.4 mn	α
Suwanee	14.9 km	α	13.9 klm	β	55.9 j–o	α	43.7 j–o	β	33.0 mno	α	38.7 l	α	2.7 n–q	α	2.6 lm	α
Tifblue	14.9 km	β	16.0 def	α	58.3 f–k	α	52.0 bcd	β	32.2 no	α	43.2 hij	β	2.8 n–q	α	3.0 jk	α
Walker	15.2 jkm	α	14.8 ijk	α	53.4 l–p	α	43.4 l–p	β	34.1 k–n	α	41.5 k	β	3.3 k–n	α	3.2 ij	α
Windy	12.5 o		–		50.5 q		–		27.3 pq		–		1.2 s		–	
Woodard	14.9 km	α	13.7 mn	β	54.3 i–p	α	41.9 nop	β	34.9 j–m	α	40.4 k	α	2.3 qr	α	2.1 n	α
Mean	16.1		15.5		59.8		47.7		36.1		43.2		4.5		4.3	
Vaccinium ashei hybrid derivatives																
Pearl River	14.9 km	α	15.1 ghi	α	53.1 k–p	α	45.0 k–p	β	32.6 mno	α	41.4 k	β	2.6 opq	α	2.6 lm	α
Pink Lemonade	14.4 mn		–		57.4 h–n		–		27.7 pq		–		3.5 kl		–	
Snowflake	–		–		–		–		–		–		–		–	
Mean	14.6		15.1		55.3		45.0		30.1		41.4		3.0		2.6	
Vaccinium corymbosum L. (northern highbush)																
Bluecrop	14.0 n	α	12.9 p	β	56.2 k–n	α	43.1 k–n	β	31.5 no	α	36.1 m	α	3.2 k–o	α	2.9 jkl	α
Duke	16.5 bcd	α	15.6 efg	α	62.2 def	α	49.2 def	β	38.3 d-h	α	45.4 def	β	3.7 jk	α	3.5 hi	α
Elliott	13.8 n	α	13.9 klm	α	52.6 nop	α	44.2 klm	β	26.9 pq	α	34.0 n	β	2.8 n–q	α	2.8 jkl	α
Mean	14.8		14.1		57.0		45.5		32.2		38.5		3.2		3.1	

*Cultivar means within year with different letters are different at the 0.05 significance level ($n = 3$).
†α and β indicate differences at the 0.05 significance level between years within a cultivar.

Table 4 Genotypic and seasonal variations of titratable acid (TA), citric acid and malic acid content from fruit of forty-two blueberry cultivars (thirty-six *V. ashei*, three *V. ashei* derivative hybrids and three northern highbush standards)

	Titratable acid (TA)				Citric acid				Malic acid			
	%				mg g^{-1} fw				mg g^{-1} fw			
Blueberry cultivar	**2008**		**2009**		**2008**		**2009**		**2008**		**2009**	
Vaccinium ashei Reade (rabbiteye)												
Alapaha	0.31q*		–		0.42 h-l		–		1.94 st		–	
Aliceblue	0.62 ghi	β†	0.68 c–f	α	0.36 i-l	α	0.40 n	α	3.85 c-g	α	4.20 cd	α
Austin	0.44 mno	β	0.54 j–n	α	0.68 fg	β	0.83 h	α	2.74 j–p	β	3.36 gh	α
Baldwin	0.74 bcd	α	0.65 e–h	β	0.12 r	α	0.11 q	α	4.21 c	α	3.69 ef	β
Beckyblue	0.45 l–o	β	0.52 l–o	α	0.27 k–q		–		2.82 j–o		–	
Black Giant	–		0.43 rs		–		0.16 p		–		2.99 jk	
Bluebelle	0.62 g–j	α	0.62 d–j	α	0.57 f–j	α	0.58 k	α	3.85 c–g	α	3.91 de	α
Bluegem	0.39 no		–		0.26 k–q		–		2.42 o–s		–	
Bonita	0.42 no		–		0.38 h–l		–		2.36 o–s		–	
Brightwell	0.44 mno	α	0.47 opq	α	0.63 fgh	α	0.67 ij	α	2.74 j–p	α	2.91 k	α
Briteblue	0.71 c–f	α	0.62 d–j	β	0.27 k–q	α	0.24 o	α	4.60 bc	α	4.06 cd	
Callaway	0.36 p	α	0.28 s	β	0.68 fg	α	0.52 l		1.92 t	α	1.47 o	α
Centurion	0.44 mno	β	0.54 j–n	α	0.76 ef	β	1.02 g	α	2.64 k–p	β	3.21 ij	α
Chaucer	0.59 g–j		–		0.18 l–r		–		3.44 fg		–	
Choice	0.51 j–m	β	0.57 i–m	α	0.62 f–i	β	0.69 ij	α	3.30 g	α	3.65 ef	α
Clara	0.48 lmn	α	0.42 rs	β	0.53 f–j	α	0.46 m	β	2.62 k–p	α	2.31 m	α
Climax	0.61 g–j	α	0.69 cde	α	1.02 d	β	1.16 f	α	3.20 g-i	α	3.64 e–g	α
Coastal	0.68 d–g	α	0.60 f–l	β	0.34 i–l	α	0.29 no	β	3.79 c–g	α	3.31 hi	β
Delite	0.52 j–m	β	0.61 e–k	α	0.50 g–k	β	0.58 k	α	2.88 j–o	β	3.39 gh	α
Early May	0.57 h–k	β	0.68 c–f	α	0.30 kl	β	0.35 no	α	3.65 fg	β	4.35 c	α
Ethel	1.14 a	α	1.03 a	β	0.70 fg	α	0.65 jk	β	5.89 a	α	5.34 a	β
Garden Blue	0.78 bc	β	0.86 b	α	0.50 g–k	β	0.55 kl	α	4.26 c	β	4.71 b	α
Homebell	0.42 no	α	0.36 rs	α	0.55 f–j	α	0.46 m	β	2.27 p–t	α	1.90 n	α
Ira	0.72 c–f	α	0.69 cde	α	0.60 f–i	α	0.58 k	α	3.77 c–g	α	3.63 e–g	α
Menditoo	0.50 klm	β	0.56 i–m	α	0.30 k–l	α	0.34 no	α	3.39 fg	α	3.80 de	α
Montgomery	0.38 op	β	0.49 m–p	α	0.45 h-l	β	0.57 k	α	2.12 r-t	β	2.72 l	α
Myers	0.54 h–l	α	0.52 l–o	α	0.39 h–l	α	0.38 n	α	3.73 c-g	α	3.58 e–g	α
Owen	0.39 no		–		0.14 m–r		–		2.73 j–p		–	
Powderblue	0.63 gh	α	0.59 g–m	α	0.26 k–q	α	0.24 o	α	4.07 c–e	α	3.77 de	α
Premier	0.56 ij	α	0.45 o–r	β	1.71 c	α	1.36 e	β	2.45 o–s	α	1.94 n	β
Satilla	1.06 ab		–		0.98 de		–		5.24 b		–	
Southland	0.67 efg	α	0.72 c	α	0.49 g–k	α	0.53 l	α	4.13 c	α	4.47 bc	α
Suwanee	0.54 h–l	α	0.58 g–m	α	0.60 f–i	β	0.65 jk	α	2.96 i–n	α	3.19 ij	α
Tifblue	0.45 l–o	α	0.49 nop	α	0.50 g–k	β	0.55 kl	α	2.78 j–p	α	3.04 jk	α
Walker	0.35 op	β	0.44 pqr	α	0.20 l-r	β	0.25 o	α	2.57 n–r	β	3.24 ij	α
Windy	0.58 g-j		–		0.34 i–l		–		3.14 g–m		–	
Woodard	1.08 ab	α	0.99 a	β	0.20 l–r	α	0.18 p	α	5.67 a	α	5.24 a	α
Mean	0.57		0.59		0.49		0.53		3.34		3.48	
Vaccinium ashei hybrid derivatives												
Pearl River	0.63 gh	β	0.73 c	α	3.74 b	β	4.35 d	α	0.57 u	α	0.66 p	α
Pink Lemonade	0.36 p		–		2.13 c		–		0.53 u		–	
Snowflake	–		–		–		–		–		–	
Mean	0.49		0.73		2.93		4.35			0.55	0.66	
Vaccinium corymbosum L. (northern highbush)												
Bluecrop	0.81 b	β	0.91 b	α	4.87 a	β	5.13 a	α	0.19 v	α	0.35 q	α
Duke	0.68 c–g	α	0.70 cd	α	4.73 a	β	4.91 b	α	0.21 v	α	0.20 q	α
Elliott	0.63 f–i	α	0.62 d–j	α	4.70 a	α	4.66 c	α	0.15 v	α	0.16 q	α
Mean	0.70		0.74		4.77		4.90		0.19		0.24	

*Cultivar means within year with different letters are different at the 0.05 significance level ($n = 3$).
†α and β indicate differences at the 0.05 significance level between years within a cultivar.

Correlation coefficients (r) between years for SSC, fructose, glucose and sucrose were 0.78, 0.71, 0.83 and 0.96, respectively (all significant at $P \leq 0.05$). In general, SSC, fructose and sucrose had values higher in 2008 than in 2009; the reverse was true for glucose. Among the 31 cultivars where samples were available for both years, the average change in rank between years for SSC, fructose, glucose and sucrose were 3.3 (range, 0–14), 3.7 (range, 0–10), 3.7 (0–13) and 1.1 (range, 0–4), respectively, thus showing that sucrose was the most stable across years among these three parameters; however, it is also a relatively minor component compared to fructose and glucose, with values only about one-tenth of either of those components (Table 3). Sugars, by virtue of their involvement with ripening might be expected to have a large environmental component.

Correlation coefficient (r) between years for TA and organic acids (citric acid plus malic acid) was 0.86 (significant at $P \leq 0.05$). Among the thirty-two cultivars where samples were available for both years, the average change in rank between years for TA, citric acid and malic acid were 3.8 (range, 0–11), 1.9 (range, 0–10) and 2.6 (range, 0–8), respectively, thus citric acid was most consistent across years (Table 4). Like sugars, acid content might be expected to have a large environmental component and might be broadly expected to behave inversely to sugars.

Howard *et al*. (2003) compared the ORAC values for blueberries in two growing seasons and found that ORAC values varied between the two years. Environmental growing conditions can also impact levels of phenolics and ORAC in blueberries. Additionally, certain genotypes vary in their capacity to synthesise phenolics under different growing conditions. Connor *et al*. (2002) also found in several highbush and interspecific hybrid blueberry cultivars grown at three locations that the antioxidant capacity, total phenolics and total anthocyanins varied considerably over two growing seasons. Kalt & McDonald (1996) found that seasonal variation in anthocyanin content among lowbush blueberry cultivars over seven seasons was quite remarkable in fruit harvested from the same site. Anthocyanin contents had shown varied by up to 2.4-fold (Kalt & McDonald, 1996).

The blueberries used in this study were collected at fully mature (100% blue) and at bush maturity from 45% to 100% with the most typical value being approximately 60%. There was no significant correlation of total anthocyanins, total phenolics, antioxidant activity, SSC, sugars, TA or organic acids with bush maturity (data not shown). Connor *et al*. (2002) also found fully mature (100% blue) blueberries harvested at two different stages of bush maturity (30–50% vs. 60–80% ripe berries on the bush) did not differ significantly for antioxidant activity.

Conclusion

This study showed that there was a wide variation in antioxidant capacity, anthocyanin content, phenolic contents, sugar and organic acid values among various blueberry genotypes and that these values vary significantly between different growing seasons. Nonetheless, relative rankings shifted only moderately between years, and it is possible to identify more desirable clones for most parameters. The diversity in the amount of these quality attributes and antioxidant capacities presents a great opportunity for genetic improvement of blueberries through breeding programmes. This study has identified several cultivars with high levels of antioxidants, and these can be used as parents for future breeding programmes. Blueberry fruit with enhanced levels of antioxidants and other nutrients will be more beneficial to human health.

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